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ACE inhibition modifies exercise-induced pro-angiogenic and mitochondrial gene transcript expression

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Skeletal muscle responds to endurance exercise with an improvement of biochemical pathways that support substrate supply and oxygen-dependent metabolism. This is reflected by enhanced expression of associated factors after exercise and is specifically modulated by tissue perfusion and oxygenation. We hypothesized that transcript expression of pro-angiogenic factors (VEGF, tenascin-C, Angpt1, Angpt1R) and oxygen metabolism (COX4I1, COX4I2, HIF-1α) in human muscle after an endurance stimulus depends on vasoconstriction, and would be modulated through angiotensin-converting enzyme inhibition by intake of lisinopril. Fourteen non-specifically trained, male Caucasians subjects, carried out a single bout of standardized one-legged bicycle exercise. Seven of the participants consumed lisinopril in the 3 days before exercise. Biopsies were collected pre- and 3 h post-exercise from the m. vastus lateralis. COX4I1 ($P = 0.03$), COX4I2 ($P = 0.04$) mRNA and HIF-1α ($P = 0.05$) mRNA and protein levels ($P = 0.01$) showed an exercise-induced increase in the group not consuming the ACE inhibitor. Conversely, there was a specific exercise-induced increase in VEGF transcript ($P = 0.04$) and protein levels ($P = 0.03$) and a trend for increased tenascin-c transcript levels ($P = 0.09$) for subjects consuming lisinopril. The observations indicate that exercise-induced expression of transcripts involved in angiogenesis and mitochondrial energy metabolism are to some extent regulated via a hypoxia-related ACE-dependent mechanism.

Exercise causes intensity-dependent vasodilation and increases blood flow to contracting muscles (Andersen & Saltin, 1985), ensuring an adequate oxygen supply to the contracting muscle. This exercise hyperemia is challenged by activation of sympatho-adrenergic and nonadrenergic vasoconstrictor systems. After mild and moderate intense exercise there is an exercise-induced inhibition of AngII-induced vasoconstriction indicating that muscle contractions attenuate adrenergic and non-adrenergic vasoconstriction. During severe exercise there is a higher sympathetic activity and an increase in the activity of plasma renin and a subsequent increase in the production of ACE and vasoconstrictor angiotensin II (AngII) is observed (Staessen et al., 1987). During increasing intensities of exercise, the concomitant increasing activity in sympathetic vasoconstrictor nerves is driven by a combination of central nervous coactivation of motor control and sympathetic outflow and muscle-derived afferents (Victor et al., 1995).

There is evidence that the role of AngII in stimulating angiogenesis relies on capillary perfusion of skeletal muscle (Petersen & Greene, 2007). AngII elevates arterial pressure because of its vasoconstrictor action and restricts blood from entering non-active muscles in order to direct blood flow to active tissues with enhanced energy turnover (Andersen & Saltin, 1985). The AngII-mediated vasoconstriction is overridden with the onset of contraction through flow-mediated dilatation of conduit arteries and arterioles and promotes angiogenesis by activating the endothelial cell population that constitutes the capillary wall (Hahn et al., 1995; Brothers et al., 2006).

Training increases the capacity for aerobic adenosine triphosphate production in skeletal muscle through an elevation in the volume density of mitochondria in untrained subjects (Hoppeler et al., 1985). After cycling exercise under lowered ambient oxygen (hypoxia), there is a larger increase in the volume density of subsarcolemmal mitochondria compared with those residing between myofibrils, indicating that local adaptations are specifically modified by the amount of oxygen in the muscle during exercise (Desplanches et al., 1993; Vogt et al., 2001; Schmutz et al., 2010). As a result, there are improvements in maximal oxygen uptake (VO_2-peak), aerobic power, fatigue resistance,

and also in subsarcolemmal mitochondria after training in hypoxia compared with normoxia (Hoppeler & Desplanches, 1992; Flück, 2006; Ponsot et al., 2006; Zoll et al., 2006).

Metabolic and vascular adaptations that may be involved in the hypoxia-specific improvement of muscle-specific aerobic capacity after repeated endurance workouts include the enzymes operating at critical biochemical steps of mitochondrial respiration under lowered muscle oxygenation during severe exercise in hypoxia (Richardson et al., 1995; Flueck, 2009). One of the key metabolic enzymes is cytochrome *c* oxidase, which is the last enzyme in the respiratory electron transport chain converting molecular oxygen to water. After one bout of intense bicycle exercise under hypoxia, increased expression of isoforms 1 and 2 of oxygen-sensitive cytochrome *c* oxidase (COX4I1, COX4I2) plays a role in the enhancements of the aerobic capacity (Flueck, 2009, 2010; Desplanches et al., 2014). After exhaustive endurance exercise in hypoxia, the expression of COX4I2 is elevated more than the isoform COX4I1 (Fukuda et al., 2007). A correlation between elevated COX4I2 transcripts post-exercise and the increased volume density of subsarcolemmal mitochondria after 30 repetitions of the exercise stimulus with training supports that transcript expression post-exercise reflects exercise-induced mitochondrial plasticity (Desplanches et al., 2014).

Another important factor, vascular endothelial growth factor (VEGF), causes increases in vascular permeability, endothelial cell proliferation, and angiogenesis. Breen et al. (1996) showed that mRNA levels of angiogenic growth factors such as VEGF in the gastrocnemius of rat were significantly increased after a bout of endurance exercise. The same was found after a period with chronic electrical stimulation during exercise (Hang et al., 1995) indicating that muscle activity leads to an increase in mRNA levels for angiogenic growth factors. The findings support the notion that transcript level alterations in skeletal muscle after a single bout of endurance exercise are a proxy for the subsequent adaptations with repetition of an endurance stimulus (Flück, 2006; Desplanches et al., 2014).

Muscle transcript expression after endurance exercise is graded with regard to metabolic stress and modulated by oxygenation (Schmutz et al., 2010). We hypothesized that the exercise-induced alteration in expression of genes of pro-angiogenic and oxygen-dependent factors are modulated by ACE activity because of its influence on muscle perfusion (Brothers et al., 2006). We hypothesized that pro-angiogenic factors such as VEGF, tenascin-C, Angpt1, and Angpt1R are increased post-exercise while oxygen-dependent factors such as COX4I, COX4I2, and HIF-1a are blunted after exercise in subjects taking an ACE inhibitor. This was tested in biopsies of knee extensor muscle within 3 h after a single bout of bicycle exercise.

Materials and methods

Experimental design

The study has been approved by the ethics board of the Manchester Metropolitan University. Written informed consent was obtained from every participant. To test our hypothesis, we carried out a pharmacological intervention with humans that were characterized anthropometrically. Fourteen Caucasian male subjects, non-specifically trained, took part in the study carrying out a maximal exercise test to characterize aerobic fitness and the muscle response to a single bout of one-legged exercise where we collected muscle and blood samples (Table 1).

Anthropometric and functional characterization

In an entry test, peak aerobic power and VO_2-peak were established with a maximal exercise test and subject's height and mass were measured. Blood pressure was measured in a calm place in seated position with a sphygmomanometer after the subject has been sitting for at least 15 min. Seven of the participants were asked to consume daily an ACE inhibitor (ACE inhibition group: lisinopril, 10 mg/day, Zestril, AstraZeneca, London, United Kingdom) in the morning for 3 days including the day of the exercise test, the other group (control group) was not asked to use the ACE inhibitor. On the testing day, subjects reported fasted to the laboratory and performed a single-leg standardized exercise test within 5 h after the last medication was consumed. Venous blood and a biopsy sample from vastus lateralis muscle were collected by a physician (S. W.). Blood collection was done via venipuncture from the upper arm before and immediately after the bout of exercise, while the subjects were in a sitting position. 5 mL of blood was drawn from the cephalic vein into a tube sprayed with dry EDTA (K2E BD Vacutainer, Belliver Industrial Estate, Plymouth, UK) placed on ice. A 2-mL aliquot was rapidly processed for the measure of AngII.

Biopsies were collected under local anesthesia (subcutaneous injection of 1 mL 2% lidocaine) before and 3 h post-exercise from the belly portion of vastus lateralis muscle with a needle device (TSK Acecut 11G, UK biopsy).

Table 1. Participants details

	ACE inhibition group	Control group
Age (years)	30.3 ± 2.7	23.3 ± 1.3
Height (m)	1.76 ± 0.04	1.80 ± 0.02
Mass (kg)	75.4 ± 4.6	72.3 ± 2.9
Exercise (h/week)	10.4 ± 1.8	3.2 ± 0.6
Before ACE inhibition		
Blood pressure (bp)		
Systolic bp (mm Hg)	130.8 ± 7.1	118.9 ± 1.8
Diastolic bp (mm Hg)	71.4 ± 4.1	71.4 ± 1.7
VO_2-peak two-legged (L/min)	3.72 ± 0.74	3.65 ± 0.32
Peak aerobic power two-leg (watt)	320.0 ± 23.5	281.0 ± 22.8
After ACE inhibition		
Systolic bp (mm Hg)	129.8 ± 4.1	
Diastolic (mm Hg)	69.6 ± 3.5	
VO_2-peak one-leg (L/min)	3.3 ± 0.2	3.2 ± 0.6
Peak aerobic power one-leg (Watt)	174.3 ± 10.9	177.0 ± 13.1
VO_2-peak one-leg vs two-leg	0.90 ± 0.02	0.86 ± 0.05
Peak aerobic power one-leg vs two-leg	0.55 ± 0.01	0.63 ± 0.02*

Values refer to mean ± SE.

*$P < 0.05$ for group difference (unpaired *t*-tests).

Maximal exercise test

Two-leg exercise aerobic capacity was measured before the one-legged test with ergospirometry during a cycling exercise test on an Excalibur bicycle (Lode, Groningen, the Netherlands). Expired air was measured breath by breath (Oxycon alpha, Jager GmbH, Wurzburg, Germany) and heart rate using a heart rate belt (Accurex Plus, Polar Electro Finland, Kempele, Finland). The test started at 40 W and subjects were asked to cycle at 80 revolutions per minute (rpm). The power was increased every 2 min with 30 W until the subject could no longer maintain a cadence higher than 60 rpm with verbal support.

One-legged exercise test

Subjects completed the one-leg exercise test with their dominant leg and the shoe taped with duct tape to the pedal on an Excalibur bicycle (Lode). The protocol of the exercise test was a 3-min warm-up at 50 W, followed by 25 min at 1.2-times body mass in watts and ending with a ramp up of 10 W/min until exhaustion (see Fig. 1 for the characteristics of the stimulus). The subjects cycled at a cadence of 80 rpm and the test ended when in the last phase the cadence dropped below 60 rpm. This protocol was chosen to maximize the metabolic stimulus for recruited muscle groups, without central aspects posed a limitation that may interact with the systemic effects of ACE activity on blood pressure and perfusion.

Assessment of AngII levels

Blood (2 mL) was withdrawn from the venous cannula into vacutainers containing 60 µL AngII inhibitor cocktail, comprising 13.35 µL of O-phenanthroline and pepstatin A in DMSO mixed with 46.65 µL of EDTA and PHMB in aqueous solution (SPI Bio, Bertin Pharma, Versailles, France). The samples were immediately centrifuged at 10 000 rpm (3000 *g*) and 4 °C for 12 min. The supernatant was separated, snap frozen in liquid nitrogen, and stored at −80 °C until analyzed. Plasma was extracted with C18 phenyl cartridge which were conditioned with 2 mL of methanol and then rinsed with 2 mL of water. Cold plasma (0.9 mL) was rapidly passed through the cartridge and subsequently washed with 1 mL of water. Absorbed angiotensins were eluted with 1 mL of methanol into conical polypropylene tubes. The eluate was evaporated to dryness by means of a nitrogen gas stream at room temperature and the residue was stored at −20 °C. AngII was assessed with the AngII enzyme immunoassay kit (Spi Bio, Montigny Le Bretonneux, France). Briefly, the plasma samples were incubated for 1 h with 100 µL of EIA buffer (reconstituted the EIA buffer vial with 50 mL of distilled water), for 5 min with 50 µL of glutaraldehyde (100 µL of glutaraldehyde diluted in 0.125 mL of concentrated wash buffer and 4.878 mL of distilled water), for 5 min with 100 µL of borane-trimethylamine (borane-trimethylamine vial diluted in 2.5 mL of 2N HCL and 2.5 mL of methanol) on a rocker platform, and with 100 µL of anti-AngII IgG tracer (reconstituted the anti-angiotensin II-IgG tracer in 10 mL of EIA buffer) at 4 °C overnight. On the next day, the samples were incubated with 200 µL of Ellman's reagent (Ellman's reagent vial in 1 mL of concentrated wash buffer and 29 mL of distilled water). The plate was run with a single quick read on an Absorbance Microplate Reader (ELx800, BIO-TEK, Swindon, UK) with wavelength 405 after 30 min, 1 h, and 2 h of incubation.

After plotting the absorbance of each standard point vs the concentration, the AngII concentrations were calculated by interpolating from this standard curve. In order to reduce variability, AngII values were normalized to the median of the pre- values.

Transcript measures

Total RNA was isolated from 10 mm^3 of biopsy samples in 20 µm sections using an RNeasy mini kit (Qiagen, cat no. 74101, Venlo, the Netherlands) and Proteinase K (Qiagen, cat no. 19131) as described by Schmutz et al. (2010). Concentration and quality of RNA were determined using a NanoDrop USV-99 AGTGene (Labgene Scientific SA, Switzerland).

cDNA was reverse transcribed from 600 ng of RNA using a OMNIscpript Kit (Qiagen, cat no. 205110) with random hexameres according to the manufactures' instructions. RT-qPCR was carried out for eight candidate genes (tenascin-C, COX4I1, COX4I2, HIF-1a, VEGF, AngPt1, AngPt1receptor, and GAPDH) and the reference gene for normalization (28S r RNA). cDNA corresponding to an estimated input of 6 ng (0.6 ng for 28 s) was dispensed into each well with a final reaction volume of 10 µL. Pooled primer pairs (200 nM each) and KAPAS Sybr FAST Master MIX 2X (Kapa Biosystems, KK4600, London, UK) were performed on Eco™ Thermal and Optical system (Illumina, Labgene Scientific SA). Primer sequences for each gene are listed in Table 2. Each sample was amplified in duplicate using the following conditions: preheating at 95 °C for 2 min, followed by 45 cycles at 95 °C for 15 s and 60 °C for 1 min. The calculation of

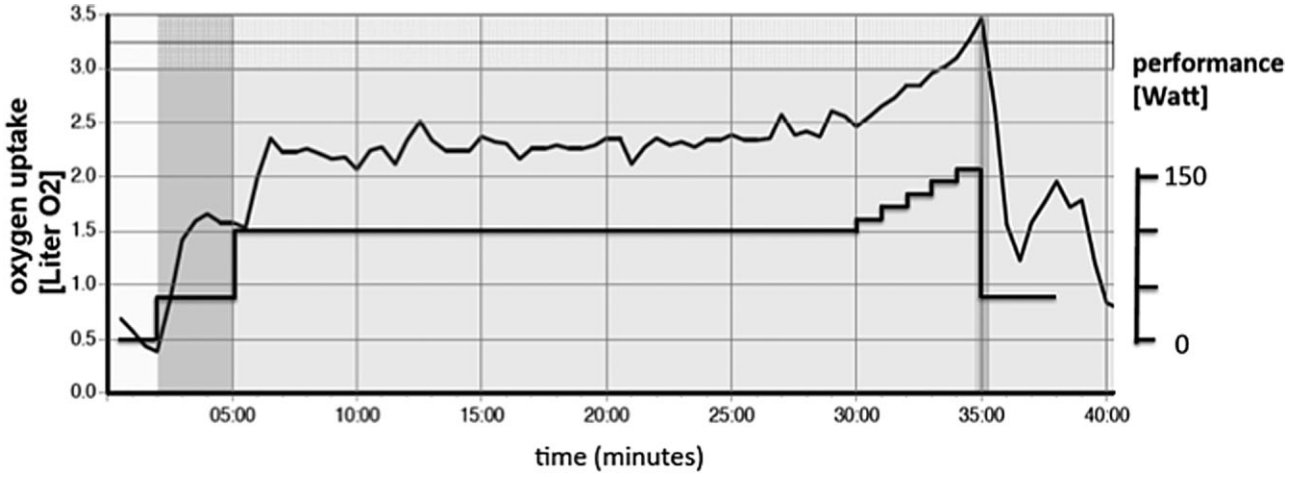

Fig. 1. The characteristics of the stimulus of the single-leg exercise test. Line graph showing the imposed performance (thick rectangular line) and oxygen uptake (thinner ragged line) of one subject over time.

Table 2. Summary of the primers sequence for the selected gene transcripts

Primer	Sequences
28S rRNA	Forward primer: 5'ATA TCC GCA GCA GGT CTC CAA-3' Reverse primer: 5'-GAG CCA ATC CTT ATC CCG AAG-3'
Angpt1	Forward primer: 5'-CGC TGC CAT TCT GAC TCA CAT A-3' Reverse primer: 5'-CGG TTA TAT CTT CTC CCA CTG TTT TC-3'
Angpt1R	Forward primer: 5'- GTT GGC CTT TCT GAT CAT ATT GC-3' Reverse primer: 5'-GGT TCT TCC CTC ACG TTT TGG-3'
COX-1	Forward primer: 5'-CTA TAC CTA TTA TTC GGC GCA TGA-3' Reverse primer: 5'-CAG CTC GGC TCG AAT AAG GA-3'
COX-4I1	Forward primer: 5'-GCC ATG TTC TTC TTC ATC GGT TTC-3' Reverse primer: 5'-GGC CGT ACA CAT AGT GCT TCT G-3'
GAPDH	Forward primer: 5'-GGA GCG AGA CCC CAC TAA CA-3' Reverse primer: 5'-GCC TTC TCC ATG GTG GTG AA-3'
HIF-1α	Forward primer: 5'-TAG TGA ACA GAA TGG AAT GGA GCA A-3' Reverse primer: 5'-TTT TTG GAC ACT GGT GGC TC-3'
Tenascin-C	Forward primer: 5'-ACCTCTCTGGAATTGCTCCCA-3 Reverse primer: 5'-CATCTGAAACTAGAAGGTTGTC-3'
VEGF	Forward primer: 5'-CAT GGC AGA AGG AGG AGG GCA GAA TCA-3' Reverse primer: 5'-ATC TTC AAG CCA TCC TGT GTG CCC CTG-3'

Angpt, angiopoetin; Bp, base par; COX, cytochrome C oxidase; GPDH, glyceraldehydes-3-phosphate dehydrogenase; HIF-1α, hypoxia-inducible factor-1a; PCR, polymerase chain reaction; VEGF, vascular endothelial growth factor.

transcript amounts were analyzed with the ΔCT method but taking the individual efficiency into account. The resulting values were standardized to those of 28S.

Protein biochemistry

Total protein homogenate was prepared from muscle biopsies. In brief, 25 µm cryosections were prepared from an estimated volume of 5 mm^3, pooled and homogenized in ice-cold 0.1 M KH2PO4 buffer including inhibitor (Complete-mini EDTA-Free reagent, Roche Diagnostics GmbH, Mannheim, Germany) using a Polytron®PT 1200E hand-held homogenizer (Kinematica AG, Lucerne, Switzerland). Protein concentration of the total homogenate was determined using the BCA method (Pierce, Rockford, Illinois, USA). 7 µg of total protein from pre-/post-sample pairs in Laemmli buffer (Bio-Rad Laboratories AG, Cressier, Switzerland) and 2% mercaptoethanol were denatured and separated by 7.5% SDS-PAGE using precast gels (Bio-Rad Mini-protean TGX Stain-free). Proteins were blotted onto a nitrocellulose membrane with a Trans-blot-Turbo Transfer System (Bio-Rad), stained with Ponceau S to record protein loading based on the staining of actin and subjected to immunodetection with specific primary (VEGF: monoclonal antibody 26503 (Abcam, Cambridge, UK); HIF-1α: monoclonal antibody GR218993 (Sigma-Aldrich Chemie AG, Buchs, Switzerland) and horse-radish peroxidase-conjugated secondary antibody in 5% milk/1% BSA – TTBS (20 mM Tris-base, 150 mM NaCl, 0.05% Tween-20, pH 7.6). Signal was recorded with enhanced chemoluminescence (Supersignal West Femto, Thermo Scientific, Hudson, NH, USA) using a PXi System (Syngene, Cambridge, UK).

Statistics

Data were organized in MS-Excel and exported into SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA) for statistical testing. Differences in blood pressure and AngII were analyzed with a paired samples *t*-test. Pre-/post-changes and fold changes in transcript and protein expression between the ACE inhibition group and control group were assessed with a multiple analysis of variance (ANOVA) and a two-factor ANOVA, respectively. Significance of a difference was declared at $P < 0.05$.

Results

Anthropometry (mass, height, age), VO_2-peak, peak aerobic power and resting blood pressure of the subjects of the two groups did not differ significantly (Table 1). There was no difference in systolic ($P = 0.16$) or diastolic blood pressure ($P = 0.94$) between the study groups. Intake of ACE inhibitor did not affect systolic ($P = 0.90$) or diastolic ($P = 0.73$) blood pressure at rest (Table 1). The ratio for peak aerobic power between the one- and two-legged VO_{2max} tests was lower for the lisinopril than the control group.

There was a trend for an effect for the intake of ACE inhibitor on the fold changes in expression of all transcripts combined post-exercise ($P = 0.09$; Fig. 2). Fold changes in transcript expression in the vastii was significantly higher 3 h post-exercise in the control group for COX4I2 ($P = 0.03$) and HIF-1α ($P = 0.05$). The level of COX4I2 was increased 3 h post-exercise in the control group ($P = 0.04$) but not in the ACE inhibition group ($P = 0.91$).

Fold changes in transcript expression post-exercise was significantly larger in the absence of ACE inhibitor intake of the pro-angiogenic transcript VEGF ($P = 0.04$). A trend for such an effect was seen for tenascin-c ($P = 0.09$).

The protein level of HIF-1α and VEGF, which transcripts were affected by ACE post-exercise were measured. HIF-1α protein was specifically increased 3 h after one-legged exercise in the group that did not consume lisinopril (Fig. 3). Conversely, VEGF protein was selectively increased 3 h after one-legged exercise in the group that did consume lisinopril.

AngII levels were 1.6 times and glucose 1.2 times increased after exercise under oral intake of ACE without significant increases in the control group (see Table 3).

Discussion

Three hours after exhaustive cycling exercise the pro-angiogenic factor VEGF and tenascin-C were increased in the ACE inhibition group, but not in the control group. This observation contrast to the results of Gavin et al. (2000b), which demonstrated that VEGF mRNA expression increased after an hour of moderate intense exercise (≈ 50% VO_{2max}) irrespective of whether the first-generation ACE inhibitor captopril was administered or not.

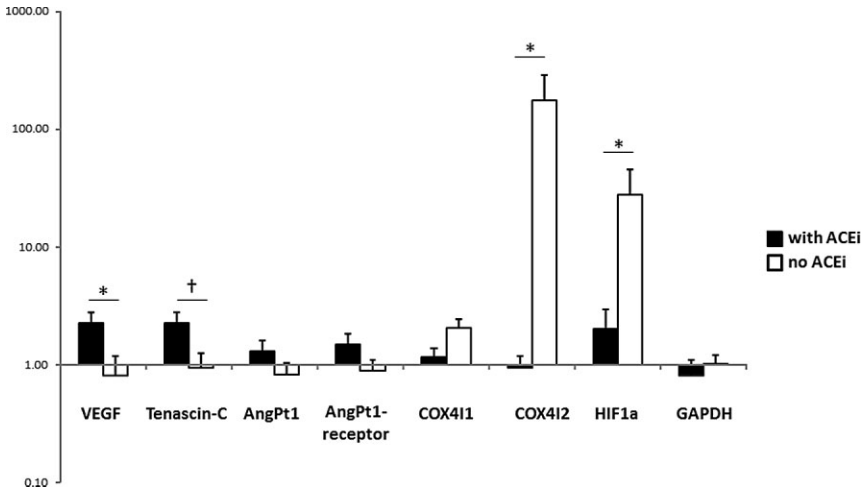

Fig. 2. Transcript expression in the vastus muscle 3 h post-exercise with or without intake of ACE inhibitor. Bar graph of the mean + SE fold changes of selected gene transcript 3 h after the single-leg exercise. AngPt1, angiopoietin 1; COX4I1, isoform 1 for subunit 4 of cytochrome c oxidase; COX4I2, isoform 2 for subunit 4 of cytochrome c oxidase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HIF-1α, hypoxia-inducible factor α-subunit; VEGF, vascular endothelial growth factor. * and † denote significant difference ($P < 0.05$) and trend ($0.05 \leq P < 0.10$) between fold changes in the group under ACE inhibition and the control group (repeated measures ANOVA).

Table 3. AngII and glucose levels with endurance exercise and the effect of ACE inhibition

	Group	Pre	Post	Fold
AngII (pg/mol)	ACE inhibition	17.4 ± 3.7	25.0 ± 3.9†	1.6 ± 0.4*
	Control	15.2 ± 4.1	19.4 ± 2.6	1.8 ± 0.6
Glucose (mmol/L)	ACE inhibition	2.3 ± 0.1	2.9 ± 0.2†	1.2 ± 0.5*
	Control	4.7 ± 0.5	4.6 ± 0.8	1.0 ± 0.2

Values are mean ± SEM paired samples *t*-test.

*Denotes $P < 0.05$ vs pre- values.

†Denotes $P < 0.05$ for post-exercise values vs the control group.

Flueck (2009) reviewed that endurance training under hypoxia enhances the structural components of local aerobic capacity by increasing mitochondrial protein expression. The low intramuscular oxygen levels in the muscle in combination with contraction-related signals are the driving force for angiogenic and mitochondrial protein expression with endurance training. Involvement of signaling to muscle remodeling with low oxygen content with exercise is illustrated by the modulation of the master regulator of hypoxia-regulated gene expression, the dimeric transcription factor HIF-1 (Semenza, 2004). Hypoxia-dependent regulation by HIF-1 is turned off in well-oxygenated conditions by the degradation of its alpha-subunit, HIF-1α. When the hypoxia drops to a critical level, HIF-1α is stabilized (Flueck, 2009). In our study, we identified that HIF-1α transcript levels were more increased in the control than the lisinopril group (Fig. 3). HIF-1α has been shown to increase after 45 min of one-legged knee-extension exercise until 6 h into recovery (Ameln et al., 2005). Our present novel evidence for the enhanced stabilization of HIF-1α in the absence of lisinopril, are in line with the former results and suggests that muscle oxygenation was more reduced in knee extensor muscle of subjects that performed the exhaustive one-legged exercise in the absence of lisinopril.

Similarly, we found that HIF-1α transcript levels were increased in the control group, while they were blunted in the ACE inhibition group. The same was observed for COX4I2. We have reported before that HIF-1α and COX4I2 transcript levels are regulated in a hypoxia-modulated manner after two-legged endurance type bicycle exercise (Desplanches et al., 2014). Thereby, COX4I2 transcript levels were increased 24 h after a single exercise bout at 65% of respective peak aerobic power output in ambient hypoxia equivalent to 4000 m above sea level. Exercise in hypoxia produces a pronounced drop in muscle oxygenation (reviewed in Desplanches et al., 2014). This emphasizes that enhanced COX4I2 mRNA expression in exercised muscle constitutes a marker of local hypoxia (Fukuda et al., 2007). These considerations support the notion based on the measure of HIF-1α protein that ACE inhibitor-induced modulation of COX4I2 and HIF-1α transcript levels in m. vastus lateralis after exhaustive endurance exercise is related to muscle oxygenation

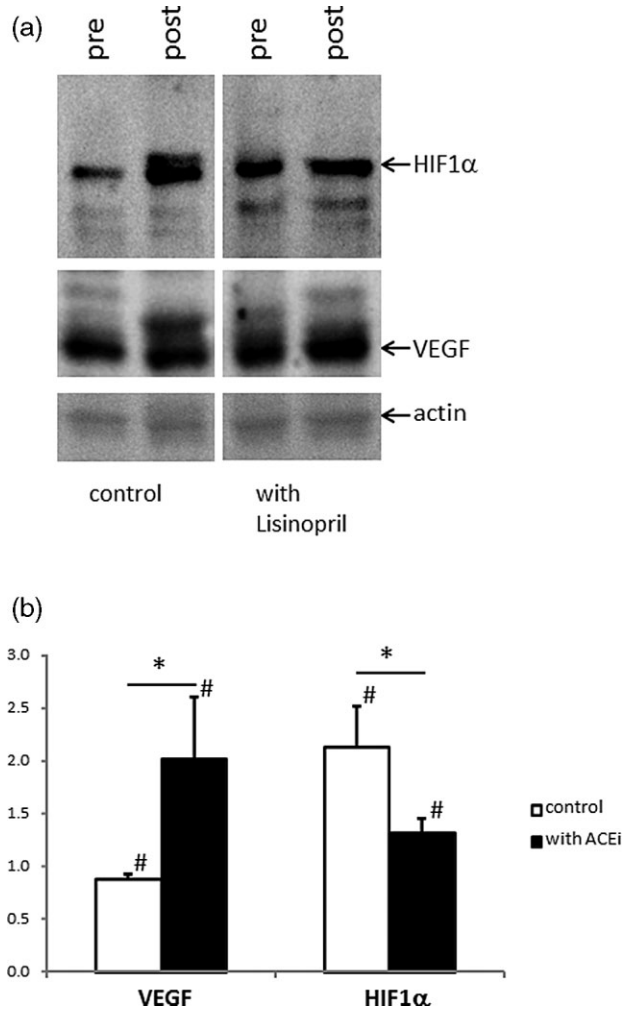

Fig. 3. HIF-1α and VEGF protein expression in function of lisinopril intake.
(a) Representative immunoblots of HIF-1α and VEGF protein pre- and 3 h post-single-leg exercise in one subject that consumed lisinopril and in a control subject. (b) Bar graph of mean + SE of fold changes in HIF-1α and VEGF protein with exercise. #$P < 0.05$ post- vs pre-exercise. *$P < 0.05$ for fold differences post- vs pre-exercise between groups (repeated). ANOVA with post-hoc test of Fisher.

during exercise. Our observations also imply that the ACE-modulated response of VEGF transcript and HIF-1α protein 3 h after one-legged exercise did not correspond (compare Figs 2 and 3). Expression of the VEGF transcript after acute hypoxia in isolated cells or chronic hypoxia in the heart (Semenza et al., 1999; Semenza, 2004) has been described to depend on HIF-1α. Under the given situation of a single measure post-exercise, it is difficult to reconcile these data with our observations. However, VEGF protein expression has been described to increase in association with muscle capillaries with enhanced contractile muscle activity (Hudlicka & Brown, 2009) while HIF-1a protein has been demonstrated to increase in nuclei and cytoplasm of muscle fibers (Ameln et al., 2005). Our data therefore indicate the possibility that differences in the oxygenation of cells involved in oxygen delivery (endothelium) and consumption (muscle fibers), and the time course of response, may provide a possible explanation for the observed difference between HIF-1α and VEGF transcript and protein expression post-exercise.

The AngII values were 1.6 times higher after exercise in the ACE inhibition group and not significantly increased in the control group. This increase in AngII is comparable with the study of Fagard et al. (1985) that demonstrated an intensity-dependent increase in angiotensin 2 after exhaustive bicycle type exercise. The increase in the ACE inhibition group, as well as the higher increase in this group compared with the control group, could indicate an incomplete inhibition of ACE during and after exercise. The difference in AngII levels between the two groups can possibly be explained by an efficient ACE inhibition at rest, reducing AngII levels to lower levels, so to allow an increase post-exercise under a possibly incomplete ACE inhibition during exercise. This is supported by the study of Aldigier et al. (1993) that found a higher increase of AngII in the ACE inhibition group during exercise compared with the control group.

We also observed a group difference in the ratio of peak aerobic power between one- and two-legged VO_{2max} tests, i.e., 0.55 vs 0.63. This ratio can be considered to reflect how peak aerobic capacity of the peripheral leg muscles can be met by the cardiovascular supply of oxygen during maximal whole-body exercise (McPhee et al., 2010). While we cannot exclude that individual differences existed in muscle perfusion between the two groups, the relatively similar and high VO_2-peak 1 vs 2-leg ratios (i.e., 0.90 and 0.86) do not support this possibility. In consequence, we suggest that our observations provide evidence for a negative impact of short duration ACE inhibition on muscle performance.

The data in the present study point at an effect of commonly used anti-hypertensive treatment on the muscle response to exhaustive bicycle type endurance exercise. This supports the view that an angiotensin-regulated mechanism dictates the hypoxia-specific gene response in peripheral muscle to endurance exercise. Gavin et al. (2000b) have previously identified that captopril reduces mRNA of the VEGF receptor Flk-1 by 30–40% in skeletal muscle independent of exercise in rat gastrocnemius muscle but did not find effects on angiogenesis-associated VEGF, VEGF receptor (Flt-1), TGF-beta(1), bFGF, and Flk-1 mRNA when assessed approximately 1 h after a one 1-h running stimulus and anaesthesia under breathing of 100% oxygen. Our findings show that the second generation ACE inhibitor lisinopril increases pro-angiogenic factors associated with the capillaries in the interstitium, i.e., VEGF and tenascin-C (Flück et al., 2000; Milkiewicz et al., 2001) and inhibits the increase in oxygen-dependent transcripts, which are thought to mainly situate in muscle fibers (COX4I2, HIF-1α). A number of factors, which

we cannot evaluate potentially, explain this difference. Differences in study design aside, this includes pronounced differences between the pharmacodynamics of the second- and first-generation ACE inhibitors lisinopril and captopril. For instance, short- and long-lived actions of lisinopril and captopril (Giles et al., 1989), respectively, likely will have added to the dissimilarity in application of captopril in the rat [20 min (Gavin et al., 2000a)] and lisinopril (3 days) in our human investigation. Lastly, the time point of measure, i.e., 1 vs 3 h after exercise, the selected transcripts, inter-species, and anatomical differences in the studied muscles may account for the discrepancy as well.

The dichotomy between transcript regulations was mirrored by the influence of lisinopril on the exercise-induced changes in VEGF and HIF-1α protein expression (Fig. 3). Our previous studies in HIF-1α-deficient transgenic mice showed that HIF-1α is involved in the up-regulation of mitochondrial gene transcripts in skeletal muscle at rest in response to the acute stimulus of severe hypoxia (Däpp et al., 2006). Consequently, we interpret the up-regulation of HIF-1α protein, HIF-1α transcripts, and mitochondrial transcripts post-exercise in subjects, which did not consume lisinopril, as evidence for hypoxia-related mitochondrial biogenesis. Conversely, we interpret the increase in VEGF mRNA and protein post-exercise in the subjects, which consumed lisinopril, to reflect a response of the capillary endothelium in exercising muscle with their assumed increased perfusion, and shear stress (Hudlicka & Brown), caused by the reduced vasoconstriction with lisinopril intake. Overall, our findings indicate that there is a shift in the activation of the gene program from muscle fibers to the surrounding interstitium under oral intake of ACE inhibitor. As ACE inhibitors in combination with endurance type of exercise are both widely prescribed for subjects with hypertension, our findings can have ramifications for improving the current anti-hypertensive treatment.

A limitation of this study is that it is not built on a cross-over design and the absence of further time-points of biopsy sampling. Both shortcomings are explained by ethical restrictions imposed for the collection of muscle material from volunteers. The absence calls for a further study to confirm our findings and expose the functional implication of our observation. As this study is based upon a single exercise bout, the protein levels corresponding to the measured transcripts (for instance VEGF) were not assessed because protein levels are expected to change as a response to endurance training rather than a single exercise bout.

Perspectives

Our findings expose that exercise-induced alterations in gene transcript and protein expression is partly under the control, or modulated, by ACE-dependent pathways. This finding addresses an aspect that has been recognized to be a most potent means of reducing the risk profile of the cardiovascular patient (Böhm et al., 2008). Awareness of the existence of such a mechanism could develop a major impact on current treatment for the hypertensive patient, which is frequently based on the inhibition of ACE activity, particularly in the situation of exercise rehabilitation when one wants to exploit the sympatholytic and angiogenic benefits of physical therapy.

Key words: Muscle, exercise, gene, angiotensin, lisinopril, hypertension, perfusion, hypoxia.

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